EFFECTS OF VINBLASTINE AND COLCHICINE ON THE SECRETION OF GLUCAGON FROM ISOLATED GUINEA-PIG ISLETS OF LANGERHANS

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1. Introduction

Glucagon secretion is thought to take place by exocytosis, a process involving movement of the glucagon-containing granules to the cell membrane, fusion of the granule membrane with the cell membrane and release of the granule contents into the extracellular space [1, 2]. A number of investigations have suggested the involvement of a microtubular-microfilamentous system in the secretion of hormones from the pancreatic B-cell [3, 4], from the adrenal medulla [5] and from the thyroid [6, 7]. It therefore seemed of interest to investigate the possible role of microtubules in the secretion of glucagon from the pancreatic A_2-cell.

In this study, we have determined the effects on glucagon release of vinblastine and colchicine, agents which cause the disruption of microtubules. It was found that both vinblastine and colchicine increased the release of glucagon from isolated guinea-pig islets of Langerhans incubated *in vitro*.

2. Materials and methods

'Velbe' vinblastine sulphate was purchased from Eli Lilly and Co. Ltd., Basingstoke, England, and colchicine was obtained from Sigma Chemical Co., Kingston, England.

The methods for isolation and incubation of islets of Langerhans, and for the radioimmunoassay of glucagon, were similar to those described previously [8]. Male guinea-pigs of Duncan-Hartley strain weighing 500–800 g were used in these experiments. To determine the effects of vinblastine or colchicine, the isolated islets were divided into two groups. One group was incubated for 60 min at 37° in medium containing glucose (5.5 mM) and either vinblastine (0.02 mM) or colchicine (0.1 mM), and the other group was incubated in medium containing glucose (5.5 mM) alone. The islets were then washed and distributed in groups of ten into small tubes containing 2 ml volumes of incubation medium. The incubation medium contained the additions shown in the table. The tubes were gassed with 95% oxygen: 5% carbon dioxide, sealed, and incubated for 30 min at 37°. Samples of the medium from each vessel were taken and stored at −20° until their glucagon content was determined by radioimmunoassay. In all experiments, samples of non-incubated medium were taken for use as blanks in the radioimmunoassay.

The rates of oxidation of [1-^{14}C] octanoic acid and of [U-^{14}C] glucose were measured using methods previously reported [9]. Isolated islets of Langerhans were incubated first for 30 min in the presence of glucose (5.5 mM) and then incubated for a period of 1 hr to determine the control rate of oxidation. Islets of Langerhans incubated first for 30 min in the presence of vinblastine or colchicine were used to determine the rates of oxidation during incubation for a further hour in the presence of these agents.

For electron microscopy, the isolated islets (after incubation) were fixed in 3% glutaraldehyde (Polysciences Ltd., Warrington, USA) in 0.05 M phosphate buffer pH 7.4, rinsed briefly, and post-fixed in 2% osmium tetroxide (Johnson Matthey Ltd., London, England) in the phosphate buffer. After dehydration in ethanol, the islets were embedded in an epoxy resin

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Table 1
Effects of vinblastine and colchicine on glucagon release from isolated islets of Langerhans.

Substances in medium	Glucagon release (ng/10 islets/30 min) Control	Control + vinblastine (0.02 mM)
5.5 mM glucose	1.51 ± 0.09 (19)	1.97 ± 0.16 (20)*
5.5 mM glucose + 5 mM octanoic acid	0.81 ± 0.06 (21)	1.31 ± 0.09 (23)*
5.5 mM glucose + 5 mM arginine	2.54 ± 0.20 (16)	3.30 ± 0.20 (17)*
	Control	Control + colchicine (0.1 mM)
5.5 mM glucose	1.27 ± 0.07 (20)	1.89 ± 0.12 (20)*
5.5 mM glucose + 5 mM octanoic acid	0.72 ± 0.07 (19)	1.25 ± 0.08 (18)*
5.5 mM glucose + 5 mM arginine	2.02 ± 0.16 (18)	2.75 ± 0.16 (18)*

*Indicates values significantly different from control ($P < 0.01$). Control incubations were preincubated in medium containing glucose alone. Glucagon release measured in the presence of vinblastine or colchicine was from isolated islets preincubated for 60 min in medium containing glucose and either vinblastine or colchicine. The number of observations is in parentheses.

by standard procedures [10]. Ultrathin sections were cut using an L.K.B. ultramicrotome and stained with a saturated solution of uranyl acetate in 50% ethanol before examination in an A.E.I. EM6B electron microscope.

3. Results and discussion

It has previously been shown that agents that interfere with microtubule function inhibit glucose-stimulated insulin release from the B-cells of the islets of Langerhans [3, 4]. In contrast, it was found that both vinblastine and colchicine caused an increase in glucagon release from islets of Langerhans incubated *in vitro* (table 1). When the basal glucagon release was stimulated in the presence of arginine, or inhibited by incubation in the presence of octanoic acid, vinblastine and colchicine still caused increases in glucagon release compared with the control values (table 1).

It has been suggested that very low levels in insulin may affect glucagon release [11, 12]. Since insulin release from isolated islets cannot be completely suppressed by agents affecting the microtubular system [3, 4], it was thought that the increase in glucagon release caused by vinblastine and colchicine in our experiments was unlikely to be mediated by changes in insulin levels in the incubation medium. However, it has been shown that a reduced production of ATP from the oxidation of fatty acids and glucose causes an increase in glucagon release from the A_2-cells [13] and it was therefore important to show that vinblastine and colchicine were not acting as inhibitors of A_2-cell metabolism. The estimation of the rates of production of $^{14}CO_2$ from radioactively-labelled octanoic acid and glucose showed that the oxidation of these metabolites was not significantly reduced in the presence of vinblastine or colchicine, so that the increases in glucagon release caused by these agents did not appear to be due to inhibition of A_2-cell metabolism.

Electron micrographs of sections from guinea-pig islets which had been incubated for 90 min in the presence of vinblastine showed the presence of crystalline material in the cytoplasm of the A_2-cells (fig. 1). No intact microtubules were observed in these cells although other organelles appeared entirely normal. In isolated islets of Langerhans incubated in the presence of glucose alone, microtubules were readily observed (figs. 2 and 3).

Our results therefore indicate that under the experimental conditions used, vinblastine and colchicine acted specifically to disrupt the microtubular system in the A_2-cells, and that one result of this disruption was an increase in the release of glucagon.

The contrast between the effects of colchicine and vinblastine on glucagon secretion reported here and their effects on insulin secretion [3, 4] further illustrates the marked differences between the control of hormone secretion from the A_2-cells and B-cells of the pancreas. Thus glucose-stimulated insulin secretion is inhibited either when the microtubular system is impaired [3, 4], in the absence of adequate concen-

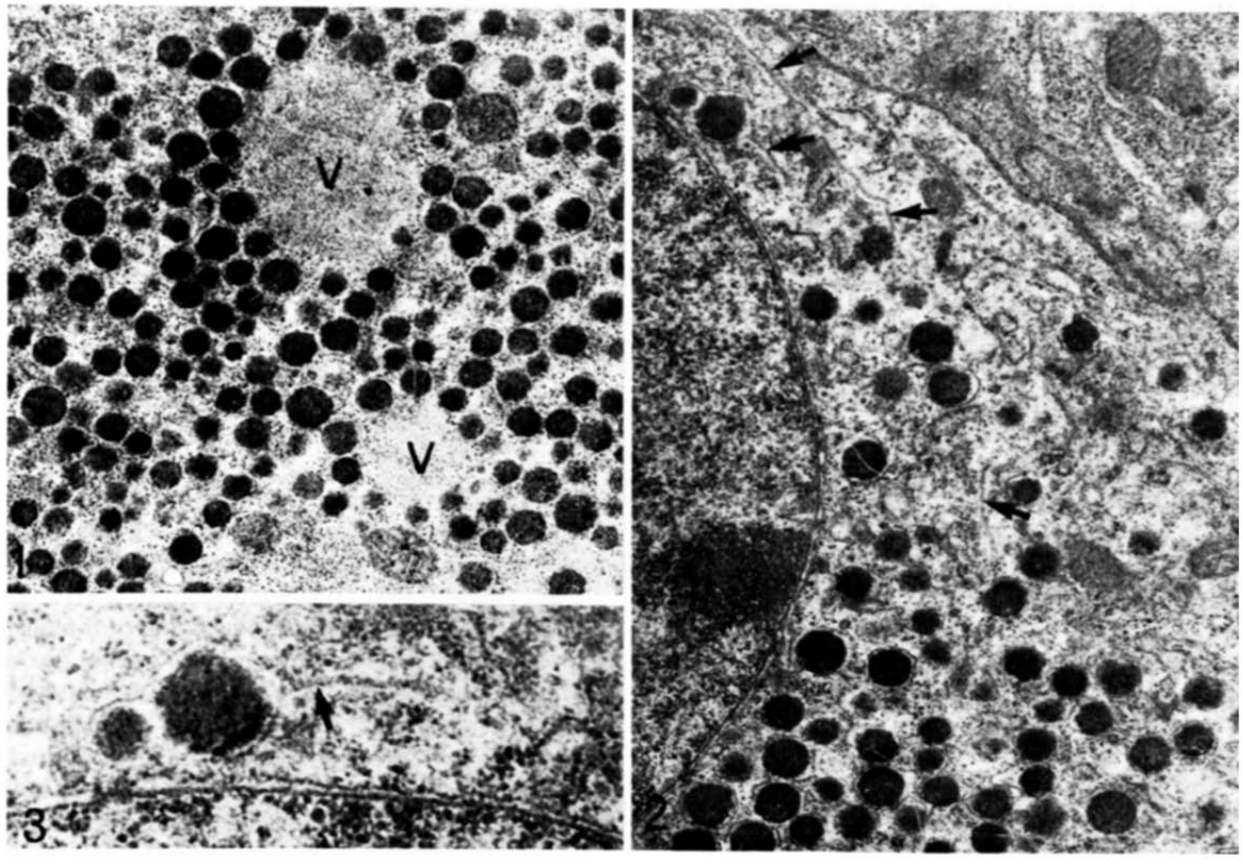

Fig. 1. Guinea-pig islet of Langerhans incubated for 90 min in the presence of 0.02 mM vinblastine. Vinblastine-induced crystals (V) have formed in the cytoplasm of the A_2-cell. Approx. magnification × 13,000.
Fig. 2. Guinea-pig islet of Langerhans incubated with glucose alone, showing the presence of cytoplasmic microtubules (arrowed) in an A_2-cell. Approx. magnification × 15,750.
Fig. 3. At higher magnification of the micrograph shown in fig. 2, a microtubule is seen in direct contact with a storage granule. Approx. magnification × 38,700.

trations of extracellular calcium [14] or when the production of ATP by the B-cell is inhibited [15]. In contrast, glucagon release from the A_2-cell increases when the microtubular system is disrupted, or when the production of ATP by the A_2-cell is reduced [13]. In addition, rates of glucagon release increase in the presence of low levels of extracellular calcium ions. In our experiments, glucagon release in the presence of glucose and in the presence of arginine increased significantly when calcium ions were removed from the incubation medium. Similar effects of calcium ion deprivation on glucagon secretion from duct-ligated pancreas pieces have recently been reported [16].

The reasons for these markedly different secretory responses of the A_2-cells and B-cells is at present unclear.

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References

[1] J. Gomez-Acebo, R. Parrilla and J.L.R. Candela, J. Cell Biol. 36 (1968) 33.
[2] A.C. Esterhuizen and S.L. Howell, J. Cell Biol. 46 (1970) 593.
[3] P.E. Lacy, S.L. Howell, D.A. Young and C.J. Fink, Nature 219 (1968) 1177.

[4] W.J. Malaisse, F. Malaisse-Lagae, M.O. Walker and P.E. Lacy, Diabetes 20 (1971) 257.
[5] A.M. Poisner and J. Bernstein, J. Pharmacol. Exp. Therap. 117 (1971) 102.
[6] P. Neve, C. Willems and J.E. Dumont, Exp. Cell Res. 63 (1970) 457.
[7] J.A. Williams and J. Wolff, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1901.
[8] J.C. Edwards, S.L. Howell and K.W. Taylor, Biochim. Biophys. Acta 215 (1970) 297.
[9] J.C. Edwards, C. Hellerstrom, B. Petersson and K.W. Taylor, Diabetologia 8 (1972) 93.
[10] W.R. Lockwood, Anat. Rec. 150 (1964) 129.
[11] K.D. Buchanan, Diabetologia 7 (1971) 473.
[12] R.H. Unger, E. Aguilar-Parada, W.A. Muller and A.M. Eisentraut, J. Clin. Invest. 49 (1970) 837.
[13] J.C. Edwards and K.W. Taylor, Biochim. Biophys. Acta 215 (1970) 310.
[14] G.M. Grodsky and L.L. Bennett, Diabetes 15 (1966) 910.
[15] H.G. Coore and P.J. Randle, Biochem. J. 93 (1964) 66.
[16] V. Leclercq-Meyer, J. Marchand and W.J. Malaisse, Proc. of the 8th Annual meeting of the European Association for the Study of Diabetes, Diabetologia, to be published.